Suppression of the Malignant Phenotype of Human Prostate Cancer Cell Line PPC-1 by Introduction of Normal Fragments of Human Chromosome 10

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INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer death among males in the United States (1-3). Previous studies have detected frequent LOH at polymorphic loci on chromosomal arms 10p and 10q in human prostate cancers. To confirm the presence of tumor suppressor genes in these chromosomal regions, fragments of normal human chromosome 10 tagged with a neomycin resistance gene were transferred into cells from a human prostate cancer cell line, PPC-1, by microcell-mediated chromosome transfer. Two of the six hybrid clones obtained showed decreased tumorigenicity in athymic nude mice and decreased efficiency of colony formation in soft agar compared with PPC-1; the other four retained fully malignant phenotypes. Analysis of polymorphic loci on chromosome 10 in these hybrid clones suggested that a tumor suppressor gene associated with prostate cancer is located within a 17-cM region at distal 10p.

RESULTS

Transfer of a normal human chromosome or a portion of the chromosome into cancer cells provides an approach to identifying tumor suppressor genes by testing the ability of normal genes to suppress the malignant phenotype. Suppression can often be achieved by introducing the normal chromosomes through microcell-mediated chromosome transfer (12-18). Recently, Dong et al. (19) used this method to identify KAI 1, a metastasis suppressor gene for prostate cancer, from chromosomal region 11p11.2. Although those experiments involved rat prostate cancer cell lines as recipients, human cancer cell lines can equally serve as recipients in microcell-mediated transfers.

We report suppression of the malignant phenotype of a human prostate cancer cell line, PPC-1, by introduction of the short arm of human chromosome 10 through microcell-mediated chromosome transfer. Structural analysis of exogenous fragments present in the suppressed hybrid clones after fusion, as opposed to nonsuppressed hybrid clones, demonstrated that a putative tumor suppressor gene for prostate cancer is located in the distal region of the short arm of chromosome 10.

MATERIALS AND METHODS

Cell Lines. The mouse-human hybrid cell HA(10)A, which was obtained from Coriell Cell Repositories, carries a human chromosome 10 tagged with a neomycin-resistance gene as its only human chromosomal material (20). This cell line was grown in DMEM with high glucose, supplemented with 10% fetal bovine serum and 500 µg/ml G418 (Life Technologies, Inc.). The recipient cells, PPC-1 (21), were grown in RPMI 1640 medium with 10% fetal bovine serum, supplemented with 1% penicillin and 1% streptomycin.

Microcell-mediated Chromosome Transfer and Fusion. Microcell-mediated chromosome transfer was performed as described by Fournier (22). The donor cell line, HA(10)A, was treated with colcemid (Sigma Chemical Co.) at a concentration of 0.06 µg/ml for 48 h. Microcells were produced following centrifugation of micronucleated cells plated on plastic bullets in the presence of 10 µg/ml cytochalasin B (Sigma) for enucleation. Microcells in a volume of 30 ml DMEM were size selected through a series of sterile filters (0.8, 0.5, and 0.3 µm) (Nucleopore) to enrich the population of microcells containing a single fragment of chromosome 10. After the filtrate was centrifuged, the pelleted microcells were resuspended with 2 ml DMEM containing 100 µg/ml phytohemagglutinin-P (DIFCO Laboratories) and allowed to attach to the monolayer of recipient PPC-1 cells for 15 min at 37°C. Fusion was accomplished by treating the cell layers with 50% (w/v) polyethylene glycol (PEG1500; Koch-light) for 1 min and rinsing with DMEM. After a 24-h incubation at 37°C, the cells were distributed 1:15 and selected in medium containing 200 µg/ml G418.

Cell Growth Studies. Cell growth studies without G418 selection were performed in triplicate by methods described previously (22).

Tumorigenicity Assay. Suspensions of 5 × 10^6 or 1 × 10^6 cells in volumes of 0.2 ml PBS were injected s.c. into 5- to 8-week-old male BALB/c athymic nu/nu mice (Charles River Breeding Laboratories). The mice were monitored regularly; tumors that formed were resected, and DNAs were extracted from them. Hybrid cell clones were judged nontumorigenic if no tumors were seen by 14 weeks after injection. All animal experiments were performed in accordance with institutional guidelines.

Analysis with SSR Markers. DNA was extracted from colonies of about 10^7 cells or from tumors resected from nu/nu mice by proteinase K-phenol-chloroform extraction (23). DNA segments that included SSR markers on chromosome 10 were amplified by the PCR essentially as described by Albertsen et al. (24). The nucleotide sequences of the primers used for PCR were obtained from the Genome Data Base. The 5’ end of one of each primer pair was labeled with [32P]ATP by polynucleotide kinase (Boehringer Mannheim). PCR products were separated on denaturing gels containing 7% acrylamide and 34% urea and autoradiographed at −80°C for 4–12 h on Hyperfilm-MP (Amersham) with an intensifying screen.

2157
includes the formation of colonies in soft agar and the formation of tumors in athymic nu/nu mice (21, 25). Furthermore, PPC-1 contains no normal copies of chromosome 10; its karyotype shows a complex translocation involving chromosomes 1, 3, and 10 (26). Through PCR analyses with polymorphic SSR markers, we have confirmed that PPC-1 is homozygous for chromosome 10 (data not shown), suggesting the loss of an entire copy of the chromosome.

Six hybrid clones (P10H1–P10H6) were obtained after fusion and G418 selection from four independent microcell experiments. To test for tumorigenicity, 5 × 10⁶ or 1 × 10⁶ hybrid cells were injected s.c. in BALB/c athymic nu/nu mice; other mice received parental PPC-1 cells. As shown in Table 1, PPC-1 formed tumors at six of six injection sites when either 5 or 1 million cells were injected, within 9 or 23 days, respectively. Similarly, four hybrid clones (P10H2, P10H3, P10H4, and P10H6) formed tumors at all injection sites when either 5 × 10⁶ or 1 × 10⁶ cells were injected, except for one site of injection with 1 × 10⁶ P10H6 cells. In contrast, P10H1 and P10H5 cells elicited tumors in one and zero of six injection sites, respectively, with 1 × 10⁶ cells/injection and only two of six or one of five injection sites, respectively, with 5 × 10⁶ cells/injection. These four tumors required much longer to develop than tumors seeded by PPC-1 cells or any of the other hybrid clones (P10H2, P10H3, P10H4, and P10H6).

To confirm suppression of the malignant phenotype, all six hybrid clones and PPC-1 cells were tested for their ability to form colonies in soft agar. As shown in Table 1, PPC-1 cells formed 541 colonies in soft agar from 1 × 10⁶ inoculated cells after 4 weeks of incubation. In contrast, H1 and H5 cells, which had shown partly suppressed tumorigenicity in nude mice, formed 15/40,000 and 5/40,000 colonies, respectively, indicating remarkably decreased efficiency of colony formation in soft agar. On the other hand, the four clones with full or almost full tumorigenicity (H2, H3, H4, and H6) formed colonies at frequencies intermediate between PPC-1 and H1 or H5 cells. The results of the two different assays indicate that the six hybrid clones fall into two groups, one group having a completely malignant phenotype and the other showing a suppressed phenotype.

Representative morphologies of the clones are shown in Fig. 1. Despite the remarkable differences of tumorigenicity and efficiency of colony formation in soft agar, no clear morphological differences are evident between a malignant clone, H2, and a suppressed clone, H1. Doubling times of suppressed versus malignant clones were not significantly different either (Table 1).

**Structural Analysis of the Fragment of Chromosome 10 Retained in Each Hybrid.** The chromosomal fragments retained in each hybrid clone were analyzed to narrow the region harboring the putative tumor suppressor gene. Highly polymorphic SSR markers mapped on chromosome 10 were used to distinguish the transferred, exogenous chromosome 10 material [from HA(10)A cells] from the chromosome 10 of PPC-1. Results of a representative PCR-based analysis using these markers are shown in Fig. 2A–E. DNA fragments containing five chromosome 10 markers (D10S526, D10S1172, D10S527, D10S508, and D10S524) all amplified from both PPC-1 and HA(10)A cells and showed length polymorphisms. In the analysis of D10S526, the most distal of these loci on 10p, DNA fragments from HA(10)A cells were amplified in all the hybrid clones (H1–H6), indicating transfer and retention of the chromosomal region contain-
**GENETIC COMPLEMENTATION OF CHROMOSOME 10 IN PROSTATE CANCER CELLS**

Fig. 2. Structural analysis of the chromosome 10 fragments transferred into each of six hybrid clones. (A–E) Analysis with SSR markers D10S526 (A), D10S1172 (B), D10S527 (C), D10S508 (D), and D10S524 (E). Sources of amplified DNA fragments, Lanes 1 to 8: PPC-1, HA(10)A, H1, H2, H3, H4, H5, and H6. F, schematic summary of results from analysis of 25 SSR markers. • retention; ○, absence of the transferred chromosomal fragment; +, suppressed clones. The order of markers and the genetic distances between them were calculated by the Utah Marker Development Group (27). Markers followed by asterisks have been assigned only within the following intervals: D10S1154, between D10S526 and D10S1153; D10S1160, between D10S504 and D10S507; D10S1169, between D10S1175 and D10S513; D10S508, between D10S507 and D10S524; D10S522, between D10S1162 and D10S1146.

In all, 14 informative SSR markers on chromosome 10 were analyzed in this way. The results are summarized in Fig. 2F, which shows that suppression of the malignant phenotype corresponds to retention of the distal region of chromosome 10p between D10S1172 and D10S506. On the basis of linkage analyses (27), the genetic distance between these two markers is, at most, 17 cM.

**DISCUSSION**

Microcell-mediated chromosome transfer is an alternative to positional cloning as a method of identifying tumor suppressor genes (18). A number of reports have demonstrated that transfer of some normal human chromosomes, tagged with selective markers, into a human or rodent cancer cell line can suppress the malignant phenotype (12–16). Even in cancer cells that carry multiple genetic changes, replacement of only one gene by a normal copy can significantly suppress the malignant phenotype (17).

In the study reported here, we judged suppression of the malignant phenotype by tumor formation in athymic nude mice and colony formation in soft agar. Two groups of hybrid clones showed remarkable differences in both assays. Although the suppression of tumorigenicity in the H1 and H5 clones was not complete, it was significant, considering that these two clones required a longer period for tumor formation than PPC-1 or its malignant derivatives, H2–4 and H6. However, clones H1 and H5 showed no remarkable changes in morphology, suggesting that the putative tumor suppressor gene they contain might not be involved in cytoskeletal systems.

Retention of the distal portion of chromosomal region 10p correlates well with the suppression phenotype in H1 and H5. Moreover, we detected the loss of the entire transferred fragment of chromosome 10 in one of the three tumors derived from H1 (data not shown). These findings indicate that a gene possessing tumor suppressor activity toward prostate cancer cell line PPC-1 is located in the distal region of 10p. On the basis of our results, and reports of frequent LOH for loci on 10p in human prostate tumors, we propose that inactivation of a tumor suppressor gene on 10p plays an important role in human prostate carcinogenesis.

Suppression of the malignant phenotype by normal human chromosome 10p has been reported in a glioblastoma cell line, U251 (28). In malignant gliomas, LOH on chromosome 10 is the most common genetic defect reported so far (29, 30). It is interesting that some epidemiological studies report a possible link between prostate cancers and glioblastoma (31). Tumor suppressor genes on 10p and 10q might both be involved in this subset of tumors.

LOH on the long arm of chromosome 10 also has been observed in
many prostate tumors (4–6). The MYC protein-regulating Mxil gene on 10p24–25 is a strong candidate for involvement, because mutations of this gene have been detected in prostate tumors that had shown LOH in the 10q region (11). In the present study, exogenous chromosome 10 was truncated in all six of the hybrid clones. That all of them contain at least part of 10p is mainly due to the fact that the neomycin resistance gene was integrated into 10p. However, the fact that none of the six hybrid clones contained 10q markers could suggest that a tumor suppressor gene on 10q is so potent that no clones containing a normal copy of that gene can grow to form colonies. Further characterization of the tumor suppressor gene on 10q and its relationship with the putative tumor suppressor in our present studies have indicated are likely to elucidate potential mechanisms of prostate carcinogenesis.

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REFERENCES


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